

REMARKS

The present application relates to inbred maize line PH48V. Claims 5-51 and 61-65 have been canceled. Claims 3, 55, 56, 58, 59, and 70 have been amended. New claims 72-75 have been added. No new matter has been added by the present amendment. Applicant respectfully requests consideration of the following remarks.

Detailed Action

A. *Status of the Application*

Applicant acknowledges the rejections of claim 54 are withdrawn.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 3, 55-61 and 63 stand rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

The Examiner rejects claims 3, 55, 58, 61 and dependents thereon as indefinite for characterizing the plant of claim 2, which lacks subsequently recited characteristics or genes, as containing additional genes or further characteristics, as stated on the bottom of page 2 of the previous Office Action.

Applicant respectfully traverses this rejection. Applicant has now amended claims 3, 55, and 58 to further characterize the trait identified and the limitation to plants that are produced from PH48V, thus alleviating this rejection. In addition, claim 61 has been canceled.

Claim 61 stands rejected as indefinite for the recitation "other than male sterility" in the last two lines.

Applicant has canceled claim 61, thereby alleviating this rejection.

In light of the above amendments and remarks, Applicant respectfully requests reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 55-61, 63, 67-69 and 70 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which is not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s) at the time the application

was filed, had possession of the claimed invention, as stated on pages 3-5 of the previous Office Action for claims 54, 57, 60-61, 63-65 and 67-69.

The Applicant traverses the rejection. Claims 5-51 and 61-65 have been canceled. Claims 55, 56, 58, 59, and 70 have been amended. New claims 72-75 have been added.

One of ordinary skill in the art would know how to cross PH48V with another maize plant. Applicant asserts it is well understood by one skilled in the art that maize is a diploid plant species thereby comprising two sets of chromosomes. The F1 hybrid seed and plant produced using PH48V, regardless of the other maize plant used, is identifiable because it will have a single set of individual maize chromosomes coming from PH48V. In addition, one of ordinary skill in the art would be able to run a molecular profile on PH48V and the F1 hybrid and be able to identify the F1 hybrid as being produced from PH48V. PH48V is a homozygous inbred plant. When the ovule or pollen is generated from this plant, it will be haploid and will contain one complete set of chromosomes from PH48V. Upon fertilization, the resulting zygote will receive one set of chromosomes from the parent inbred plant resulting in the diploid zygote. Inbred PH48V has a unique set of genes present on its chromosomes and this unique set is also present in the hybrid.

As stated in the specification on page 15, lines 2-29, there are many laboratory-based techniques available for the analysis comparison and characterization of plant genotype such as Restriction Length Polymorphisms (RFLPs) and Simple Sequence Repeats (SSRs). Such techniques may be used to identify whether or not PH48V was used to develop a hybrid. Any person of skill in the art could run a molecular profile of PH48V based upon the deposit Applicants have made. Therefore, it would be routine to one of ordinary skill in the art to run the profile of a hybrid plant and determine whether or not PH48V was used as a parent.

Claims 61 and 63 have been canceled. Claims 55, 56, 58, 59, and 70 have been amended and are to methods of making a maize plant through the utilization of PH48V. Applicant points out that anyone of skill in the art would know how to utilize the well established methods disclosed in the specification with PH48V. Description of such occurs throughout the specification and descriptions can also be found in introductory plant breeding books. As stated in the written description guidelines, an old process performed with a novel material is novel in and of itself. 66 Federal Register 1099, Vol. 66, No. 4 (January 5, 2001).

The Examiner acknowledges on page 5 of the Office Action, that "backcross breeding is well known in the art". Further, as stated in Openshaw *et al.* submitted herewith, "[t]he backcross breeding procedure has been used widely to transfer simply inherited traits into elite genotypes. ... Today, backcrossing is being used to transfer genes introduced by such techniques as transformation or mutation into appropriate germplasm." Openshaw *et al.* further notes breeders, by using molecular markers, may obtain a very high degree of genome identity between the backcross conversion and the recurrent parent after two backcrosses. See Marker-assisted Selection in Backcross Breeding, Openshaw, S.J. *et al.* Marker-assisted selection in backcross breeding. In: Proceedings Symposium of the Analysis of Molecular Data, August 1994, pages 41-43. Crop Science Society of America, Corvallis, OR (1994) included as Appendix A. The backcross method has been successfully used since the 1950's (see pages 585-586 of Wych, 1988 included in the Information Disclosure Statement). Thus, Applicants assert such mutant genes or transgenes may be introgressed into elite lines such as PH48V without undue experimentation. As further evidence of this, Poehlman *et al.* (1995) on page 334, submitted in the Information Disclosure Statement, states that, "[a] backcross-derived inbred line fits into the same hybrid combination as the recurrent parent inbred line and contributes the effect of the additional gene added through the backcross." In addition, Wych (1988) on page 585-86, discusses how the male sterility trait is routinely backcrossed into an inbred line and how this is used to produce a sterile/fertile blend of an F1 hybrid in order to reduce seed production costs. In fact, many commercial products are produced in this manner, and those of ordinary skill in the art consider the F1 hybrid produced with the male sterile (backcross conversion) inbred to be the same variety as the F1 hybrid produced with the non-backcross conversion inbred.

The Examiner also states that the morphological and physiological traits of PH48V progeny are not described. The test of written description does not require a morphological and physiological description. Rather, it is whether subject matter was described in such a way to convey to one of ordinary skill in the art that the inventor had possession of the claimed invention. While PVP is distinct from patents, the scope of protection conferred by PVP provides a clear indication that breeders of ordinary skill in the art consider F1 hybrids, backcross conversions and transgenic conversions to be within the scope of the invention of the variety itself. See previously submitted Appendix B. These derivatives, variants and closely related progeny easily and routinely created through the use of this newly developed line are

encompassed within the scope of the invention of the variety itself. The fact that the progeny have not been created does not prevent them from being protected in this manner. As stated in MPEP § 2163(3)(a), "An invention may be complete and ready for patenting before it has actually been reduced to practice."

The Examiner rejects claims to transgenic PH48V plants and PH48V plants comprising single gene conversions. Newly amended claims 55, 56, 58, 59, 70 and new claims 74-75 have been amended or written to claim specific traits conferred by mutant genes or transgenes, which include the traits of insect resistance, herbicide resistance, disease resistance, and male sterility. Applicant respectfully points out that examples of transgenes, mutant genes, genes, and traits that can be introduced into the PH48V are given in the application on page 20, lines 16-34, and also on page 21, line 34, through page 34, line 2. The Examiner suggests that the claims be amended to include a list of transgenes. In order to expedite prosecution claims 55, 56, 58, 59, 70 are newly amended and new claims 74-75 list the type of traits that may be conferred. It is unclear to Applicant how the Examiner's rejections pertained to claim 70 as Applicant asserts claim 70 is a method claim utilizing a deposit in a well known process that is patentable pursuant to the written description guidelines. Nevertheless it should be noted that PH48V comprising a mutant gene or a transgene, even if it is for a transcription factor, is distinct from another inbred line comprising that same mutant gene or transgene and still retains the benefit of Applicants' invention.

Claims 55-61, 63 and 70 stand rejected under 35 U.S.C. § 112, first paragraph as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains or with which it is most nearly connected, to make and/or use the invention, as stated on pages 5-7 of the last Office Action for claims 61 and 64-65.

Applicant respectfully traverses this rejection. The Applicant has provided assurance that at least 2500 seeds of inbred maize line PH48V have been deposited with the ATCC. In view of this assurance, the rejection under 35 U.S.C. § 112, first paragraph, should be removed. (MPEP § 2411.02). Further, Applicant has canceled claims 61 and 63 and amended claims 55, 56, 58, 59, and 70, thereby alleviating this rejection.

In addition, Applicant submits a patent application "need not teach, and preferably omits, what is well known in the art." *Hybritech Inc. v. Monoclonal Antibodies Inc.*, 802 F.2d 1367, 231 U.S.P.Q. 81 (Fed. Cir. 1986); MPEP § 601. One of ordinary skill in the art of plant breeding

would know how to evaluate the traits of two plant varieties to determine if there is no statistically significant variation when determined, for example, at a 5% significance level and when grown in the same environmental conditions between the traits expressed by those varieties. Applicant claims progeny produced by backcrossing with PH48V and retaining phenotypic characteristics of PH48V. Distinguishing identifying characteristics in the chemical and biotechnological arts, dealing with DNA, are those such as: partial structure, physical and/chemical properties, functional characteristics, known or disclosed correlation between structure and function, method of making, and combinations of the above. In plants, phenotypic characteristics are identifying characteristic correlated with DNA structure. It is respectfully submitted that Applicants' claims are sufficiently enabled and described by the specification.

In light of the above amendments and remarks, Applicant respectfully requests reconsideration and withdrawal of the rejections to claims 55-61, 63, and 67-70 under 35 U.S.C. § 112, first paragraph.

Summary

Applicant acknowledges that claims 1, 2, 4, 52-54, 66 and 71 are allowed.

Applicant acknowledges that the claims remain free of the prior art, given the failure of the prior art to teach or suggest an inbred maize plant having all the morphological and physiological characteristics of the exemplified corn plant, or having essentially all of the characteristics plus an additional introgressed trait, or methods of their use. This clearly indicates that inbred maize line PH48V as a whole is considered to be distinguishable from the prior art for the purposes of novelty and non-obviousness. Therefore Applicant respectfully submits that with the deposit of the representative seed of PH48V the rejections under 35 U.S.C. § 112, first paragraph are improper and request reconsideration and withdrawal of these rejections.

Conclusion

In conclusion, Applicant submits in light of the above amendments and remarks, the claims as amended are in a condition for allowance, and reconsideration is respectfully requested. If it is felt that it would aid in prosecution, the Examiner is invited to contact the undersigned at the number indicated to discuss any outstanding issues.

No fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,



LILA A. T. AKRAD, Reg. No. 52,550
McKEE, VOORHEES & SEASE, P.L.C.
801 Grand Avenue, Suite 3200
Des Moines, Iowa 50309-2721
Phone No: (515) 288-3667
Fax No: (515) 288-1338
CUSTOMER NO: 27142

Attorneys of Record

- pw/bja -

Marker-assisted Selection in Backcross Breeding

S.J. Openshaw

Pioneer Hi-Bred Intl. Inc., P.O. Box 1004, Johnston, IA 50131

S.G. Jarboe¹

CIMMYT, Lisboa 27, Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico

W.D. Beavis

Pioneer Hi-Bred Intl. Inc., P.O. Box 1004, Johnston, IA 50131

Abstract. The backcross breeding procedure has been used widely to transfer simply inherited traits into elite genotypes. Genetic markers can increase the effectiveness of backcrossing by 1) increasing the probability of obtaining a suitable conversion, and 2) decreasing the time required to achieve an acceptable recovery. Simulation and field results indicated that, for a genome consisting of ten 200-cM chromosomes, basing selection on 40 or 80 markers in 50 BC individuals that carry the allele being transferred can reduce the number of backcross generations needed from about seven to three.

The backcross breeding procedure has been used widely to transfer simply inherited traits into elite genotypes. Usually, the trait being transferred is controlled by a single gene, but highly heritable traits that are more complexly inherited have also been transferred successfully by backcrossing; for example, maturity in maize (Rinke and Sentz, 1961; Shaver, 1976). Today, backcrossing is being used to transfer genes introduced by such techniques as transformation or mutation into appropriate germplasm.

Several plant breeding textbooks give good descriptions of the backcross procedure (Allard, 1960; Fehr, 1987). A donor parent (DP) carrying a trait of interest is crossed to the recurrent parent (RP), an elite line that is lacking the trait. The F_1 is crossed back to the RP to produce the BC₁ generation. In the BC₁ and subsequent backcross generations, selected individuals carrying the gene being transferred are backcrossed to the RP. The expected proportion of DP genome is reduced by half with each generation of backcrossing. Ignoring effects of linkage to the selected DP allele being transferred, the percentage recurrent parent (%RP) genome expected in each backcross generation is calculated as:

$$\%RP = 100 [1 - (0.5)^{n+1}]$$

where n is the number of backcrosses.

Backcrossing of selected plants to the RP can be repeated each cycle until a line is obtained that is essentially a version of the RP that includes the introgressed allele. After six backcrosses, the expected recovery is >99% (Table 1).

Until recently, discussions of the recovery of the RP genome during backcrossing have emphasized the expected values for

%RP shown in Table 1, and have largely ignored the genetic variation for %RP that exists around the expected mean. With the development of genetic markers capable of providing good genome coverage, there has been interest in taking advantage of that variation to increase the efficiency of backcrossing.

Selection for RP marker alleles can increase greatly the effectiveness of backcross programs by allowing the breeder to 1) select backcross plants that have a higher proportion of RP genome, and 2) select backcross individuals that are better conversions near a mapped donor allele being transferred (i.e., select for less linkage drag). Expressed in practical terms, using genetic markers to assist backcrossing can 1) increase the probability of obtaining a suitable conversion, and 2) decrease the time required to achieve an acceptable recovery.

Issues to consider when planning a marker-assisted backcross program include 1) the time advantage of using markers to assist backcrossing, 2) the number of markers needed, and 3) the number of genotypes to evaluate. In this report, we use results from previous literature, computer simulation, and empirical studies to provide some guidelines.

Table 1. Expected recovery of recurrent parent (RP) genome during backcrossing, assuming no linkage to the gene being transferred.

Generation	%RP
F_1	50.0000
BC ₁	75.0000
BC ₂	87.5000
BC ₃	93.7500
BC ₄	96.8750
BC ₅	98.4375
BC ₆	99.2188
BC ₇	99.6094

¹Formerly with Purdue University, West Lafayette, Ind.

Materials and methods

The maize genome was the model for the simulation. The simulated genome contained ten 200-cM chromosomes. Simulation of crossing over was based on a Poisson distribution with a mean of 2.0 ($\lambda = 2$) (Hanson, 1959), which, on average, generated one cross over for every 100-cM length. The simulations reported here assume no interference. Codominant genetic markers were evenly distributed in the genome and sites of the donor gene were randomly assigned to genome locations.

Simulations were conducted with the following parameters:

Number of progeny: 100 or 500.

Backcross generations: BC₁, BC₂, and BC₃.

Number of markers: 20, 40, 80, or 100.

Number selected to form the next BC generation: 1 or 5.

Selection was based on 1) presence of the donor allele and 2) high %RP. %RP was calculated as the average of the (one or five) selected individuals. Values presented are the mean of 50 simulations.

Results

In the computer simulation study, all methods modeled greatly increased the speed of recovering the RP genome compared to the expected recovery with no marker-assisted selection (compare Tables 1 and 2). At least 80 markers were required to recover 99% of the RP genome in just three BC generations (Table 2). Use of at least 80 markers and 500 progeny allowed recovery of 98% RP in just two BC generations. Response to selection was diminished only slightly by spreading the effort over five selections. Using markers, the number of backcross generations needed to convert an inbred is

reduced from about seven to three.

By the BC₃ generation, there appears to be no practical advantage to using 500 vs. 100 individuals. If the presence of the donor trait in the backcross individuals can be ascertained before markers are genotyped, then only half the number of individuals indicated in the tables will need to be analyzed.

When a small number of markers are used, they quickly become non-informative; i.e., selection causes the marker loci to become fixed for the RP type before the rest of the genome is fully converted (Table 3; Hospital et al., 1992). This situation was most prominent in the larger populations, where a higher selection intensity placed more selection pressure upon the marker loci. Accordingly, it is of interest to consider how closely the estimation of %RP based on markers reflects the actual genome composition. The combination of estimation of %RP based on fewer markers and subsequent selection tends to bias the estimates upward (compare Tables 2 and 3).

The results from the simulation compare well with real field data. In a typical example, 50 BC₃ plants carrying the gene being transferred were genotyped at 83 polymorphic RFLP loci (note that this corresponds to a population size of 100 unselected plants in Tables 2 and 3). The five best BC₃ recoveries had estimated %RP values of 85.9%, 82.7%, 82.0%, 81.4%, and 81.2%. After evaluating 10 BC₃ plants from each selected BC₃, the best BC₃ recovery had an estimated %RP of 94.6%.

Discussion

The simulations (Table 2; Hospital et al., 1992) and our experience indicate that four markers per 200-cM chromosome is adequate to greatly increase the effectiveness of selection in the BC₃. However, using only four markers per 200 cM will likely make it very difficult to map the location of the gene of interest. Adequate summarization of the data is an important

Table 2. Percent recurrent parent genome during marker-assisted backcrossing.

Generation	100 Progeny				500 Progeny				
	No. markers				No. markers				
	20	40	80	100		20	40	80	100
<i>One selected</i>									
BC ₁	84.5	84.5	84.2	88.0	89.9	90.7	90.2	90.5	
BC ₂	95.0	95.2	95.8	97.2	96.3	97.7	98.3	98.6	
BC ₃	97.4	97.6	98.9	99.2	97.7	98.3	99.4	99.5	
<i>Five selected</i>									
BC ₁	82.9	85.1	84.9	84.7	87.7	88.1	88.9	88.9	
BC ₂	93.7	95.0	95.8	95.7	95.5	96.8	97.8	97.9	
BC ₃	97.1	98.3	98.8	98.9	97.3	98.5	99.3	99.3	

Table 3. Estimates of percent recurrent parent genome, based on marker loci.

Generation	100 Progeny				500 Progeny				
	No. markers				No. markers				
	20	40	80	100		20	40	80	100
<i>One selected</i>									
BC ₂	98.7	97.8	95.6	97.2	100.0	99.1	98.6	98.0	
BC ₃	100.0	99.8	99.3	99.5	100.0	100.0	99.9	98.2	
<i>Five selected</i>									
BC ₂	96.4	96.5	96.2	95.8	100.0	98.5	98.3	98.2	
BC ₃	99.9	99.8	99.3	99.1	100.0	100.0	99.9	99.8	

part of a marker-assisted backcross program. Ideally, the markers used can supply data that can be represented as alleles of loci with known map position. Estimation of %RP, mapping the position of the locus of interest, and graphical display of the results (Young and Tanksley, 1989) are all useful in understanding and controlling the specific backcross experiment being conducted.

It appears that, with the use of genetic markers, the portion of the RP genome that is not linked to the allele being transferred can be recovered quickly and with confidence. The recovery of RP will be slower on the chromosome carrying the gene of interest. A considerable amount of linkage drag is expected to accompany selection for the DP allele in a backcross program. For a locus located in the middle of a 200-cM chromosome, the length of the DP chromosome segment accompanying selection is expected to be 126, 63, and 28 cM in the BC₁, BC₂, and BC₃ generations, respectively (Hanson, 1959; Naveira and Barbadilla, 1992). Our observations support the recommendation of Hospital et al. (1992) that preference be given to the selection for recombinants proximal to the allele of interest, but that selection for recovery of the RP elsewhere in the genome also be considered. This two-stage selection can probably be done quite effectively ad hoc by the breeder once the data is adequately summarized; however, Hospital et al.

suggest ways to incorporate the two criteria into a selection index such that each component of selection is assured appropriate weighting.

Use of genetic markers can greatly increase the effectiveness of backcrossing, and they should be used in any serious backcrossing program if resources are available to the breeder.

Literature Cited

- Allard, R.W. 1960. Principles of plant breeding. Wiley, New York.
Fehr, W.F. 1987. Principles of cultivar development: v. I. Theory and technique. Macmillan, New York.
Hanson, W.D. 1959. Early generation analysis of length of heterozygous chromosome segments around a locus held heterozygous with backcrossing or selfing. *Genetics* 44:843-847.
Hospital, F., C. Chevalier, and P. Mulsant. 1992. Using markers in gene introgression breeding programs. *Genetics* 132:1199-1210.
Rinke, E.H. and J.C. Senz. 1961. Moving corn-belt germplasm northward. *Ann. Hybrid Corn Industry Conf.* 16:53-56.
Shaver, D.L. 1976. Conversions for earliness in maize inbreds. *Maize Genet. Coop. Newslett.* 50:20-23.
Young, N.D. and S.D. Tanksley. 1989. Restriction fragment length polymorphism maps and the concept of graphical genotypes. *Theo. Applied Genet.* 77: 95-101.